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Effect of Adding Various Organic Acids During the Feed Withdrawal Period on *Salmonella* Reduction in Broilers

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Abstract: *Salmonella* contamination of broilers is a major problem that faces the poultry industry in Kuwait and elsewhere since it affects the consumption of poultry meat. Therefore, utilization of different control measures leading to the reduction of *Salmonella* contamination is an important task for the broiler industry and the public health authorities in Kuwait. An important strategy is to significantly reduce the levels of *Salmonella* at the farm level and improve the manufacturing practices in the processing plant to prevent the risk of cross contamination. In our Department, different treatments have been used to control the contamination of this pathogen at the farm level, one of which is presented in the current study. The objective of the current study is to determine the effect of using different organic acids in the drinking water during the feed withdrawal period on reducing *Salmonella* in broilers. One hundred and twenty broiler chicks were originally housed in each of 36 floor pens. At the time of feed withdrawal, the pens were divided into four groups of nine pens each and were used for one of four treatments. These treatments included the control group and received untreated water, the second group received water containing 0.1% acetic acid, the third group received water containing 0.1% formic acid and the fourth group received water containing 0.1% lactic acid for a period of eight hrs. This study was repeated both in the summer and winter seasons. The prevalence of *Salmonella* on the chicken body, ceca and in the crop was determined before and post treatment at the farm and then at the processing plant. In addition to reducing body *Salmonella* contamination significantly ($p<0.05$) post treatment at the farm, in both seasons, it was found that acid treatments, in the summer season, significantly ($p<0.05$) reduced *Salmonella* contamination in the carcass at the processing plant from 36% (control) to 16, 13, 13% for acetic, formic and lactic acid treatments, respectively. In the winter season, both formic and lactic acid treatments reduced *Salmonella* contamination in the carcass at the processing plant and the reduction was significant ($p<0.05$) for formic acid treatment. It can be concluded that using organic acids in the water during the feed withdrawal period, both in the summer and winter seasons, can be beneficiary in reducing broiler *Salmonella* contamination both at the farm and at the processing plant.

Key words: Broilers, organic acids, *Salmonella*

INTRODUCTION

Salmonella contamination of broilers is a major problem that faces the poultry industry in Kuwait and elsewhere since it affects the consumption of poultry meat. In addition, *Salmonella* food poisoning associated with the consumption of poultry products also is a continuous problem for the local poultry industry. Therefore, utilization of different control measures leading to the reduction of *Salmonella* contamination is becoming an extremely important issue for both the broiler industry and the public health authorities in Kuwait. *Salmonella* is one of the major contributors to food-borne illness linked to the poultry products like eggs and poultry meat (Parker *et al*., 2006). During the recent years, the poultry producers in Kuwait suffered major economic losses because of the lack of an effective pathogen reducing-monitoring program for poultry. In addition, on two occasions in 2006, the Kuwait Ministry of Public Health banned locally produced broilers from the market because of the high incidence of *Salmonella* contamination (Al-Zenki *et al*., 2009).

In Kuwait, a need exists to quantify the situation regarding the incidence of *Salmonella* in poultry and to implement methods to prevent and control this pathogen in the farm and at the processing plant.

The U.S. Department of Agriculture (USDA) has proposed that all slaughter establishments should apply at least one antimicrobial treatment or other approved intervention procedure to livestock and poultry carcass (Nissen *et al*., 2001; Dincer Baysal and Unluturk, 2005).

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It should be mentioned that different methods have been used to reduce *Salmonella* in the farm and at the processing plant such as drug therapy and vaccination (White *et al*., 1997), competitive exclusion (Stavric, 1987; Salvat *et al*., 1992; Stavric, 1992; Seo *et al*., 2000; Al-Zenki *et al*., 2009) and only drug therapy (Seo *et al*., 2000). In addition, organic acids have been used to reduce *Salmonella*. These organic acids, dips or spray, such as acetic, lactic, citric and propionic acids either individually or combined, are beneficial in controlling undesired microorganisms on refrigerated meats (Bell *et al*., 1986; Hamby *et al*., 1987; Lillard *et al*., 1987; Marshall and Kim, 1996). In addition, use of certain lactic acid bacteria as a probiotics has been proposed for many years. These probiotics bacteria have been shown to prevent enteric disease, as well as, improve the overall health of poultry (Tellez *et al*., 2006; Wolfenden *et al*., 2007). Furthermore, it was found that feeding chickens with acidified feed reduced the PH in the crop, which resulted in a reduction in horizontal transmission of *Salmonella* (Thompson and Hinton, 1997).

Organic acids have been investigated because of their bactericidal activity and they are generally recognized as safe and therefore are utilized for preservation in many food applications (Quartey-Papafio *et al*., 1980; Izat *et al*., 1989; Dickson and Anderson, 1992).

The mechanism of biocidal activity of acids is not quite known, however, it was suggested that *Salmonella* does not have ability to reduce intracellular acids and therefore, causes a lethal accumulation of acid anions within the cell (Van Immerseel *et al*., 2006; Jarquin *et al*., 2007).

Further research is needed to compare the effect of different organic acids on reduction of *Salmonella* both at the farm and at the processing plant. Therefore, in the current research, we are reporting on the effect of adding organic acids during the feed withdrawal period on *Salmonella* reduction in broilers. In this experiment, commercial products for reducing *Salmonella* in broilers were evaluated using three different organic acids to study whether these treatments would reduce *Salmonella* contamination on the body, carcass, ceca and in the crop.

MATERIALS AND METHODS

Bird management: The bird management of the current experiments is similar to what was previously reported (Al-Zenki *et al*., 2009). Briefly, broiler chicks (Indian River x Indian River), obtained from one commercial hatchery were used in the current study. Our study was conducted on a commercial farm using one part of a house. This part of the house was divided into 36 floor pens that were used in the current experiment as will be described below. These pens were equipped with nipple drinkers, received continuous lighting and wood shavings were used as floor bedding. Un-medicated corn/soy-based diets that met the NRC requirements (NRC, 1994) were provided *ad libitum*. The chicks received a pre-starter diet (24.4 CP, 3, 029 kcal ME/kg) from hatch until 7 days-of-age, a starter diet (22.5 CP, 3036 kcal ME/kg) from 8 days till 21 days-of-age and a finisher diet (21.6 CP, 3171 kcal ME/kg) from 22 days till 35 days-of-age when the experiments ended and the birds were slaughtered. It is important to note that all experiments were conducted in a manner that avoided unnecessary discomfort to the animals by the use of proper management and laboratory techniques.

Experimental design: In this experiment, 36 pens mentioned above was measured at 5 m^2 per pen and housed one hundred and twenty chicks providing 0.042 m^2/bird. The 36 pens were divided into four groups of nine pens each. Each nine pens were assigned to one of the four treatments that will be described below, at 35 days of age. Data from each pen was considered as one value and thus providing nine replicates per treatment. This study was conducted during the months of August and September (summer season) and the average temperature in the house throughout the period was 28°C. The experiment was repeated during the months of December and January (winter season) and the average temperature in the house throughout the period was 23°C. This was done in order to determine whether or not the effects of different organic acids on reducing *Salmonella* contamination in the farm and at the processing plant, is influenced by season. In all, a total of 8,640 birds were used for the entire study.

Treatments: The treatments included the control group which was provided with sterile double distilled water, the second group received water containing 0.1% acetic acid, the third group received water containing 0.1% formic acid and the fourth group received water containing 0.1% lactic acid, all these treatments were provided at 5 weeks of age during the feed withdrawal period which was about eight hours. Each treatment had nine replicates as mentioned previously. This study was repeated both in the summer and winter seasons.

Sample collection and microbiological analysis: Samples were collected both from the farm and at the processing plant. Farm samples from the whole body, ceca and crop before and after treatment were collected at 35 days-of-age. While at the processing plant samples were collected I) at the evisceration step to obtain the ceca and crop samples and ii) post-chilling step to obtain the carcass samples.

The prevalence of *Salmonella*, which is defined as the occurrence or presence of *Salmonella*, on the chicken body and in the ceca and crop was determined as described previously (Al-Zenki *et al*., 2009). Briefly, five randomly selected chickens were removed from each of

the nine pens that were assigned for each treatment and killed by cervical dislocation. Following the detection of *Salmonella* in the collected samples, as will be described below, the percent contamination within each pen was considered as one value (one replicate) thus providing nine replicates from each treatment which were used for the statistical analyses.

The whole bird's body and the carcasses were placed in large sterile stomacher bags containing 400 mL of 0.1% buffered peptone water and rinsed by shaking for two minutes. The whole bird and carcasses rinse solution was then poured into sterile containers. The body surface was then rubbed with a sterile cloth containing 70% ethanol alcohol to disinfect the area where the ceca and crops were to be removed and then aseptically dissected. Ceca and crop were removed and their contents diluted 1:3 with 0.1% buffered peptone.

Samples of whole bird and carcass rinses, crop and ceca contents were pre-enriched at 37°C for 24 hr in 0.1% buffered peptone water. After incubation, 1 mL of the pre-enriched samples were transferred to 9 mL of tetrathionate broth and selenite cysteine broth, respectively and incubated for 24 h at 37°C. Following incubation, samples were then streaked onto xylose lysine deoxycholate and bismuth sulphite agar. Suspected *Salmonella* colonies were stabbed into triple sugar iron agar and lysine iron agar slants and presumptive *Salmonella* were confirmed by serotyping. Prevalence of *Salmonella* from the five birds that were tested from each pen provided the percent *Salmonella* contamination in each pen. The percentages for the nine pens for each treatment provided the nine replicates and these replicates were used for the statistical analyses.

Data analysis: Data were analyzed using a one-way analysis of variance utilizing the S-plus statistical program (Crawley, 2002). The treatments within each season were the main effect either before or after adding the organic acids. Means were separated using Tukeys test and significance was set at $p<0.05$.

RESULTS AND DISCUSSION

Effects of different treatments on reduction of *Salmonella* prevalence during summer and winter seasons at the farm: Data on effects of adding organic acids during feed withdrawal period on reduction of *Salmonella* prevalence at the farm on the whole body, ceca and crop are shown in Table 1, 2, 3 respectively.

Table 1: Effect of organic acid treatments during feed withdrawal period on *Salmonella* prevalence on the body, at the farm during summer and winter seasons at the farm

	Season							
	Summer				Winter			
	Treatment							
	Control	Acetic acid	Formic acid	Lactic acid	Control	Acetic acid	Formic acid	Lactic acid
Before treatment	44*±24[a]	33±14[a]	33±14[a]	33±17[a]	15.6*±9[a]	13.3±14[a]	26.7±26[a]	24.4±13[a]
After treatment	50±26[a]	16±19[b]	13±20[b]	0±00[b]	50.0±11[a]	15.6±11[b]	13.3±11[b]	0.0±09[b]

[a/b]Means within a season in a raw with no common supscripts differ significantly ($p<0.05$). *Values are expressed as means±SD (n = 9)

Table 2: Effect of organic acid treatments during feed withdrawal period on *Salmonella* prevalence on the ceca, at the farm during summer and winter seasons at the farm

	Season							
	Summer				Winter			
	Treatment							
	Control	Acetic acid	Formic acid	Lactic acid	Control	Acetic acid	Formic acid	Lactic acid
Before treatment	31.1*±23[a]	22.2±23[a]	15.6±17[a]	20.0±14[a]	15.6*±13[a]	15.6±9[a]	13.3±14[a]	8.9±11[a]
After treatment	33.3.2±23[a]	13.3±20[b]	11.1±15[b]	20.0±23[a]	33.3±10[a]	13.3±7[b]	11.1±10[b]	20.0±07[ab]

[a/b]Means within a season in a raw with no common supscripts differ significantly ($p<0.05$). *Values are expressed as means±SD (n = 9)

Table 3: Effect of organic acid treatments during feed withdrawal period on *Salmonella* prevalence on the crop, at the farm during summer and winter seasons

	Season							
	Summer				Winter			
	Treatment							
	Control	Acetic acid	Formic acid	Lactic acid	Control	Acetic acid	Formic acid	Lactic acid
Before treatment	33.3*±24[a]	28.9±18[a]	20.0±10[a]	17.8±12[a]	0*±0	0±0	0±0	0±0
After treatment	33.3±21[a]	13.3±20[a]	15.6±19[a]	8.6±11[b]	0±0	0±0	0±0	0±0

[a/b]Means within a season in a raw with no common supscripts differ significantly ($p<0.06$). *Values are expressed as means±SD (n = 9)

Our results showed that, in general, *Salmonella* prevalence in the body, ceca and crop in all groups before treatments was less in the winter season than in the summer season. This seasonal difference could be due to more stress imposed on the birds in the summer season than in the winter season. Stress-associated conditions have been shown to increase shedding of *Salmonella* Enteritidis in chickens (Holt, 1992; Holt, 1993; Holt, 2003). Furthermore, McBride *et al*. (1978) have reported greater frequency of *Salmonella* detection in flocks reared in the summer compared with the winter season.

It is clear from our results that all acid treatments significantly ($p<0.05$) reduced *Salmonella* prevalence in the body at the farm in both summer and winter seasons. Cox *et al*. (1994) found that broilers supplemented with organic acids led to reduced levels of *Salmonella* in the intestine which will result in reduction in the spread of *Salmonella* in the broiler house. This indicates that acid treatments at the farm is important and would lead to reduction of *Salmonella* at the farm.

As to the effect of acid treatments on the prevalence of *Salmonella* in the ceca, it was found in the current study, that *Salmonella* prevalence was reduced significantly ($p<0.05$) when acetic acid and formic acid were used, however, that was not the case for the lactic acid treatment. The results are the same in both summer and winter seasons.

These results could indicate that both acetic acid and formic acid have stronger effects on reduction of *Salmonella* prevalence than lactic acid at least as it relates to *Salmonella* reduction in the ceca. This difference in the action could be due to the different characteristics of organic acids that could affect its biocidal activity as reported by Hsiao and Siebert (1999). This implies that not all acids have similar effects on reducing the *Salmonella* prevalence in broilers. It is important to mention that the mechanism of biocidal activity of acids is not quite known, however, it was suggested that *Salmonella* does not have ability to reduce intracellular acids and therefore, causes a lethal accumulation of acid anions within the cell (Van Immerseel *et al*., 2006; Jarquin *et al*., 2007). Furthermore, it has been reported that organic acids exhibit at least two distinct modes of action with respect to their antimicrobial properties, namely pH reduction of the environment and direct anti-microbial property of un-dissociated acid molecular (Jordan *et al*., 2009).

Contrary to the effects on the ceca, lactic acid was the only acid that reduced *Salmonella* prevalence in the crop to 8.6% compared to 33.3% for the control in the summer season, however, the difference was significant at ($p<0.06$). Organic acids can be effective in reducing *Salmonella enteritidis* in the crop as previously reported (Byrd *et al*., 2001).

It should be mentioned that none of the acid treatments had any effects on *Salmonella* prevalence in the crop during the winter season. This is obvious since the *Salmonella* prevalence was zero in the crop of all groups including the control group. Therefore, it can be concluded that utilizing organic acids during the feed withdrawal period at the farm can result in reducing *Salmonella* prevalence on the broiler body regardless to the season. However, the effects on the ceca or crop could depend on the acid that is used.

Effects of different treatments on *Salmonella* reduction during different seasons at the processing plant: Data on effects of using organic acids during feed withdrawal period on *Salmonella* prevalence on the carcass, ceca and crop at the processing plant are shown in Table 4.

Our results showed that all acid treatments, during the summer season, significantly ($p<0.05$) reduced *Salmonella* prevalence on the carcass at the processing plant. Our results agree with the findings of Byrd *et al*. (2001); Byrd *et al*. (2003); Van Immerseel *et al*. (2006); Wolfenden *et al*., (2007); Lilly *et al*. (2011); Jarquin *et al*. (2007), who reported that direct acidification of the water with organic acids could significantly reduce the amount of recoverable *Salmonella* on broiler carcass. Reducing *Salmonella* contamination in broiler carcasses is extremely important and significant since carcasses are the final products that reach the consumer. Hence, our findings emphasis the important role that organic acids play in reducing *Salmonella* contamination in the broiler final product that reaches the consumers. Therefore, it can be recommended to use organic acids in the water during the withdrawal period for broilers.

It should be mentioned that during the winter season, only formic acids had significantly ($p<0.05$) reduced *Salmonella* contamination in the carcass. This indicates that formic acid might be more effective as a treatment to reduce broiler *Salmonella* contamination in both summer and winter seasons. Thormar *et al*. (2006) and Lawhon *et al*. (2002) reported that the effect on *Salmonella* contamination differs within each organic acid treatment and each organic acid has a unique effect on bacteria that normally is present in the crop and gastrointestinal tract (Thormar *et al*., 2006; Lawhon *et al*., 2002). Characteristic of organic acids such as chain length, side chain composition, pkA values and hydrophobicity could be factors that affect the biocidal activity of the acid (Hsiao and Siebert, 1999). It has been reported that formic acid has the lowest molecular weight when compared with other organic acids (Jordan *et al*., 2009).

As to the effect of adding organic acids in the water during the feed withdrawal period, in the present study, on *Salmonella* prevalence in the ceca and crop at the processing plant during either summer or winter

Table 4: Effect of organic acid treatments during feed withdrawal period on *Salmonella* prevalence on the carcass, ceca and the crop at the processing plant during summer and winter seasons

	Season							
	Summer				Winter			
	Treatment							
	Control	Acetic acid	Formic acid	Lactic acid	Control	Acetic acid	Formic acid	Lactic acid
Carcass	36.0±13[a]	16.0±17[b]	13.0±10[b]	13.0±10[b]	31.4±17[a]	31.1±25[a]	8.6±21[b]	20.0±10[ab]
Ceca	20.0±17[a]	11.1±23[a]	8.9±11[a]	8.9±15[a]	20.0±16[a]	11.1±15[a]	5.7±10[a]	11.1±15[a]
Crop	33.3±33[a]	11.1±15[a]	11.1±15[a]	22.2±25[a]	0.0±0	0.0±0	0.0±0	0.0±0

[a/b]Means within a season in a raw with no common supscripts differ significantly ($p<0.05$). *Values are expressed as means±SD (n = 9)

season, there were no significant differences ($p>0.05$) between any of the treatments and the control group. Our results disagree with the findings of Jarquin *et al*. (2007) who reported that direct acidification of the water with organic acids could reduce the amount of recoverable *Salmonella* in the crops and ceca when used during the pre-slaughter feed withdrawal period. The differences in the results could be due to the fact that Jarquin *et al*. (2007) used a mixture of organic acids and not a single one like in the current study. This could indicate that the use of a mixture of organic acids could be needed in some cases to reduce the Salmonella contamination in crops and ceca. It is important to note that *Salmonella* prevalence for both the ceca and crop of the group that was treated by formic acid was less than the other groups, even though the difference was not significant. Again this could indicate that formic acid might be more effective as a treatment to reduce broiler *Salmonella* contamination. Furthermore, as mentioned previously the most important issue is to reduce *Salmonella* contamination in the carcasses since it is the final product that reaches the consumers and not the crop or the ceca.

It can be concluded from our results that using organic acids in the water during the feed withdrawal period, both in the summer and winter seasons, can be beneficiary in reducing broiler *Salmonella* contamination both at the farm and at the processing plant.

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